

Amendments to the Specification:

Please replace the paragraph at page 29, lines 14-26 with the following replacement paragraph:

This reaction can be assayed in order to characterize the enzyme or identify specific inhibitors of its activity in a number of ways:

1. Measurement of the formation of Met-tRNA^{Met} can be specifically determined using radiolabelled methionine and separating free methionine from Met-tRNA using precipitation/filtration techniques (e.g. in cold trichloroacetic acid^{1,2} (Calender & Berg 1996 Biochemistry 5:1681-90; Toth MJ & Schimmel P 1990 J. Biol. Chem. 265:1000-04))
2. The full acylation reaction can also be measured by analyzing production of either PPi or AMP which are produced in stoichiometric ratio to the tRNA acylation. This may be achieved in a number of ways, for example using colorimetric³ (Hoenig 1989 J. Biochem. Biophys. Meth. 19:249-52); or enzyme coupled⁴ (Webb TM 1994 Anal. Biochem. 218:449-54) measurement of Pi after addition of excess inorganic pyrophosphatase or using enzyme coupled assays to directly measure AMP or PPi production⁵ (Sigma Chemicals Catalog, 1986).
3. The partial reaction (a) can be assayed through radiolabel isotopic exchange between ATP and PPi, since each of the steps in this part of the reaction are freely reversible. This reaction typically has a k_{cat} around 10-fold higher than the full acylation reaction (a+b), and is readily measured using chromatographic principles with separate PPi from ATP (i.e. using activated charcoal^{1,2} (Calender & Berg 1996 Biochemistry 5:1681-90; Toth MJ & Schimmel P 1990 J. Biol. Chem. 265:1000-04)).

Please replace the paragraph at page 29, lines 28 to page 30, line 18 with the following replacement paragraph:

It is also possible to define ligand interactions with MRS in experiments that are not dependent upon enzyme catalysed turnover of substrates. This type of experiment can be done in a number of ways:

1. Effects of ligand binding upon enzyme intrinsic fluorescence (e.g. of tryptophan). Binding of either natural ligands or inhibitors may result in enzyme conformational changes which alter enzyme fluorescence. Using stopped-flow fluorescence equipment, this can be used to define the

microscopic rate constants that describe binding. Alternatively, steady-state fluorescence titration methods can yield the overall dissociation constant for binding in the same way that these are accessed through enzyme inhibition experiments

2. Spectral effects of ligands. Where the ligands themselves are either fluorescent or possess chromophores that overlap with enzyme tryptophan fluorescence, binding can be detected either via changes in the ligand fluorescence properties (e.g. intensity, lifetime or polarisation) or fluorescence resonance energy transfer with enzyme tryptophans. The ligands could either be inhibitors or variants of the natural ligands (i.e. fluorescent ATP derivatives or tRNAMet labelled with a fluorophore).

3. Thermal analysis of the enzyme:ligand complex. Using calorimetric techniques (e.g. Isothermal Calorimetry, Differential Scanning Calorimetry) it is possible to detect thermal changes, or shifts in the stability of MRS which reports and therefore allows the characterisation of ligand binding.

References

- ~~1. Calender & Berg (1966) Biochemistry 5, 1681-1690~~
- ~~2. Toth MJ & Schimmel P (1990) J. Biol. Chem. 265, 1000-1004~~
- ~~3. Hoenig (1989) J. Biochem. Biophys. Meth. 19, 249-252~~
- ~~4. Webb TM (1994) Anal. Biochem. 218, 449-454~~
- ~~5. Sigma Chemicals Catalogue, 1986~~

Example 3 Aminoacylation Assays for MRS Activity.